was to analyze the impact of CsA levels in the development of grade 2-4 aGVHD in the setting of allo-RIC in the first four weeks after transplantation.

Patients and Methods: We included 156 consecutive patients [64 (41%) women], median age 52 (17-69) years, who underwent HLA-identical sibling allo-RIC at a single institution. RIC included fludarabine 150 mg/m2 plus busulfan 10 mg/kg (for myeloid malignancies n = 53) or melphalan 70-140 mg/m2 (lymphoid malignancies n = 103). GVHD prophylaxis was based on CsA plus methotrexate (MTX) (n = 121, 78%) or mychopenolate mofetil (MMF) (n = 35, 22%). CsA levels were measured at least twice weekly during the first four weeks (or until discharge) and the dose was adjusted to maintain blood levels between 200 and 300 ng/ml.

Results: As the use of MTX vs MMF did not impact on the incidence of grades 2-4 aGVHD patients were analyzed together. The median blood concentrations of CsA at 1st, 2nd, 3rd and 4th weeks after allo-SCT were 134 (95 CI:10-183), 219 (95 CI: 54-261), 253 (95 CI: 53-314) and 224 ng/ml (95 CI:30-411) respectively. The number of patients who were in the optimal range in the 1st, 2nd, 3rd and 4th weeks after allo-RIC were 34/150 (22%), 92/154 (59%), 86/148 (58%) and 53/123 (56%). Sixty six patients developed grade 2-4 aGVHD for a cumulative incidence of 42% (95% CI 35-51%) at a median of 38 (range:18-138) days after allo-SCT. In univariate analysis the variables associated with a higher incidence of 2-4 aGVHD were: male sex ($p = 0.016$), female to male donor-recipient sex combination ($p = 0.05$), and median CsA levels in the second ($p = 0.02$) and third ($p = 0.02$) weeks. In multivariate analysis, the only significant variables associated with higher 2-4 aGVHD were female to male donor-recipient sex combination (HR 2; $p = 0.01$) and the median CsA levels in the third week (HR 0.097, $p = 0.039$).

Conclusion: The levels of CsA in the immediate post-transplant period were suboptimal in almost 50% of patients. Low levels of CsA were associated with higher incidence of grade 2-4 aGVHD. A more stringent monitoring and modification of CsA in the early phase post-Allo-RIC may be helpful to prevent aGVHD.

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ANALYSIS OF THE FLT3-ITD AND NPM1 MUTATIONS IN AML PATIENTS WITH INTERMEDIATE RISK RECEIVING ALLOGENEIC STEM-CELL TRANSPLANTATION

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Background: Chromosomal abnormality is the most important prognostic factor for AML patients. Recently, prognosis of cytogenetically normal AML patients has been reported to be affected by the presence of fms-like tyrosine kinase 3 gene internal tandem duplication (FLT3-ITD) and nucleophosmin 1 gene (NPM1) mutations. In the present study, we analyzed retrospectively the FLT3-ITD and NPM1 mutations in AML patients with cytogenetically intermediate risk who received allo-SCT and we evaluated the effect of the mutations on the outcome of allo-SCT.

Patients and Methods: 23 patients (11 males and 12 females) with a median age of 46 years (range: 27-65) receiving allo-SCT between 2005 and 2009 whose BM samples were available were enrolled in this study. Allo-SCT consisted of 10 matched siblings, 8 matched unrelated donors and 5 unrelated cord blood. GVHD prophylaxis included CsA/ short term MTX (10) or FK/short term MTX (13). Genomic DNA was extracted from PBMC and amplified by PCR using specific primers. Analysis of FLT3-ITD or NPM1 exon 12 mutations was carried out by either electrophoresis or direct DNA sequencing.

Results: FLT3-ITD mutation was found in 5 patients (21.7%). FLT3-ITD mutation occurred only in cytogenetically normal patients (positivity: 38.5%). On the other hand, NPM1 mutation was found in 6 patients (26.1%). Acute GVHD above grade II was found in 4 patients (57.1%) with FLT3-ITD mutation while only 3 patients (20.0%) without FLT3-ITD mutation manifested the complication. Frequency of acute GVHD above grade II was 33% and 41% in NPM1 mutation positive and negative patients, respectively. All the patients with FLT3-ITD mutation manifested relapse of the disease following allo-SCT while relapse occurred in 5 patients (33.3%) without FLT3-ITD mutation. However, there was no marked difference in relapse rate between patients with or without NPM1 mutation (33% vs. 41%). Median period of overall survival was 0.184 and 1.619 years in FLT3-ITD mutation positive and negative patients, respectively.

Conclusions: It was demonstrated that the rate of both relapse and acute GVHD was significantly higher in AML patients with FLT3-ITD mutation compared to FLT3-ITD mutation negative patients. NPM1 mutation exerted minimal effect on the incidence of acute GVHD and relapse of the disease. These results suggested that allo-SCT patients with cytogenetically intermediate risk can be stratified to poor prognosis group if FLT3-ITD mutation is identified.

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PLASMA CYTOKINE PROFILES AT DAY ZERO: MYELOABLATIVE CONDITIONING EXHIBITS A MORE INFLAMMATORY PROFILE THEN REDUCED INTENSITY CONDITIONING IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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While myeloablative conditioning (MAC) has been the conventional preparative regimen for allogeneic stem cell transplant, reduced intensity conditioning (RIC) has increasingly been used, especially in non-malignant conditions. MAC has been associated with a cytokine storm that may contribute to graft versus host disease (GVHD) while RIC has shown lower tissue damage which may lead to a lower release of inflammatory cytokines. We hypothesized that patients receiving MAC would express a more inflammatory subset of plasma cytokines on Day Zero compared to patients receiving RIC.

METHODS: We prospectively collected samples on 52 consecutive consented patients who underwent allogeneic transplantation at Cincinnati Children's Hospital Medical Center between December 2007 and October 2008. Blood samples were collected at Day 0. Patient Characteristics are in Table 1.

Table 1.

	RIC	MAC
Number of Patients	23	29
Patient Age -median (range) years	6.7 (0.6-17.9)	8.1 (0.8-19.4)
Patient Gender	13 males/10 females	20 males/9 females
Diagnosis	23 non-malignant	12 malignant/17 non-malignant

A Bio-Plex Pro Assay was used to measure plasma levels of GM-CSF, G-CSF, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ, MCP-1, MIP-1b, TNF-α, IL-18, and MIF. Plasma concentrations of sTNF-R1 were measured by ELISA. Soluble IL-2 Receptor alpha (sIL2Rα) levels were measured using the Immulite platform.

RESULTS: Wilcoxon rank sum test was used to compare the plasma cytokine levels between the RIC and MAC groups. IL-6, G-CSF, sIL-2Rα, IL-17 and IL-7 plasma levels were found to be different in the two groups ($p \leq 0.05$). We additionally analyzed 2 groups within the RIC cohort-10 patients received distal alemtuzumab (between Days -22 and -13 pretransplant) and 12 patients received proximal alemtuzumab (between Days -12 and -8 pretransplant). In this analysis, patients who received distal alemtuzumab have higher levels of the tested cytokines including IL-1b, IL-6, IL-8, IFN-γ, MIF and TNF-α ($p \leq 0.05$).

DISCUSSION: A pro-inflammatory cytokine profile (increased IL-6, G-CSF and sIL-2Rα) is seen in MAC patients when compared to RIC patients who have increased levels of differentiation (IL-17) and growth (IL-7) cytokines. The timing of alemtuzumab prior to transplant affects the cytokine profile on Day 0. Patients receiving distal alemtuzumab have higher levels of the pro-inflammatory cytokines

(IL-1b, IL-6, IL-8, IFN-γ, MIF and TNF-α). We have observed clinical differences in the rates of mixed chimerism and GVHD depending on when the alemtuzumab is given. This data suggests that the cytokine milieu may contribute to the development of GVHD and Transplant Related Morbidity (TRM).

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OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN COMMUNITY CANCER CENTERS: SINGLE INSTITUTION EXPERIENCE

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Variability in outcomes after hematopoietic stem cell transplantation (HCT) due to differences in health care delivery is traditionally referred to as "center effect". Data analysis by CIBMTR demonstrated improved day 100 survival after related donor (RD) HCT with greater physician involvement in patient's care regardless of medical school affiliation. We hypothesized that the greater physician involvement in patient's care at our community transplant center would compensate for the lack of infrastructure available to academic centers and result in comparable outcomes. We retrospectively reviewed the medical records of 50 consecutive patients who underwent matched unrelated (MUD) HCT (n = 26) or RD HCT (n = 24) for hematological malignancies between August 2007 and April 2010. GVHD prophylaxis used was Tacrolimus/Methotrexate or Tacrolimus/Mycophenolate. MUD HCT recipients recieved ATG in addition. Twenty one (42%) and twenty eight patients (56%) of cohort had progressive/persistent disease and high risk cytogenetics at time of transplant respectively. Thirty three patients (66%) had Charlson Co-morbidity index of 3 or more. Patients characteristic is shown in the table below.

Table 1. Patients characteristic

Number of patients	50 (100%)
Age	Median 56 (Range 23-71)
Patients above the age of 55	27 (54%)
Patients below the age of 55	23 (46%)
Match related (RD)	23 (46%)
Mismatched related 5/6	1 (2%)
Matched unrelated (MUD)	22 (44%)
Mismatched unrelated	4 (8%)
Male	30 (60%)
Female	20 (40%)
Diagnosis	
AML/MDS	26 (52%)
ALL	8 (16%)
Myelofibrosis	2 (4%)
CLL	2 (4%)
T-cell prolymphocytic leukemia	2 (4%)
CML (accelarated phase)	1 (2%)
HD	2 (4%)
Severe aplastic anemia	1 (2%)
Non Hodgkins lymphoma	3 (6%)
Multiple myeloma	3 (6%)
Stem cell source	Peripheral stem cells 50 (100%)
Status at transplant	
Complete remission -1	20 (40%)
Complete remission-2	9 (18%)
Progressive disease	7 (14%)
Persistent disease	14 (28%)
Prior Transplants	
Autologous	7; non tandem (14%)
Allogeneic related	5 (10%)
Cytogenetics	
High Risk	28 (56%)
Normal	19 (38%)
Not available	3 (6%)
Charlson Comorbidity Index	
Score 0	7 (14%)
Score 1-2	10 (20%)
Score 3-4	20(40%)
Score 5 and above	13 (26%)
Conditioning Regimens	
Full Intensity (FIC)	20 (40%)
Reduced Intensity(RIC)	21(42%)
Non Myeloablative (NMA)	9 (18%)

GVHD, graft versus host disease; OS, overall survival; DFS, disease free survival; NRM, non relapse mortality; NMA, Flu/TBI, RIC, FluBU-2/Flu-Mel, FIC FluBU-4/BUCY/CYTBI

OS at 100 days and 1 year were 86% and 67% respectively. There was no statistical difference in OS between RD and MUD; (83% vs. 88% at day 100 and 74% vs. 64% at 1 year for RD and MUD recipients respectively, $P = 0.85$). DFS was 55% at 1year. Again, there was no statistical significance difference in DFS between RD and MUD at 1 year ($P = 0.48$). The cumulative incidence of relapse was 16% at 1 year (21% for RD and12% for MUD). We found no difference in the cumulative incidence of NRM between RD and MUD recipients at day 100 (12%). In contrast, NRM was higher at 1 year in MUD recipients of 34% vs. 25% for the RD recipients. The overall cumulative incidence of acute GVHD grade II-IV was 47.8% with incidence of severe GVHD grade III/IV of 16%. The cumulative incidence of chronic GVHD was 67.6%.

Conclusions: Allogeneic HCT outcomes in the community seem to be comparable to outcomes reported in literature. In this single institution experience, despite the absence of direct cause and effect relationship, the greater involvement of physicians in the patient's care may have contributed to the improved outcomes in this high risk cohort of patients. Community transplant centers may contribute in the future to meet the increased demands for allogeneic HCT with reasonable outcomes.

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PRELIMINARY RESULTS OF PHASE II TRIAL OF CLOFARABINE WITH PARENTERAL BUSULFAN (CLO/BU) FOLLOWED BY ALLOGENEIC RELATED OR UNRELATED DONOR TRANSPLANTATION FOR THE TREATMENT OF HEMATOLOGIC MALIGNANCIES

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BACKGROUND: RIT regimens are common, but relapse remains a problem. We proposed and tested a mid-intensity regimen using clofarabine (CLO) with busulfan (BU). We hypothesized this combo would be well tolerated and offer greater anti-leukemic efficacy than existing RIT regimens.

METHODS: We enrolled 20 patients on this single IST, with AML (10), ALL (1), CLL (1), MDS (2) and MDS-AML (6). 15 patients had prior therapies. The regimen was: CLO 40mg/m2 iv daily x5, BU 3.2 mg/kg iv daily x2, followed by 1 rest day, followed by HSCT. Donors were matched at A, B, C, DR, DQ using DNA SBT or mid-res DNA typing. Mismatch ≤ 1 antigen was allowed. GVHD prophylaxis was FK506 and MTX 5mg/m2 iv (d 1,3,6).

RESULTS: Endpoints included toxicity, engraftment, incidence/severity of AGVHD, and disease response.

All patients experienced grade 4 hem tox. Median time to ANC recovery (18/20 patients used GCSF) was 13 days (d9 - d17). Engraftment (> 80% donor chim. at d30) occurred in all patients by FISH and/or STR. Selected tox. included; 2 patients - hand/foot syndrome (1 Gr. 3, REL.); 1 resp. failure (Gr. 3 poss. REL) resolved completely; 5 patients - elevated ALT/AST (Gr.3-4, REL) resolving at regimen completion; other tox. were ≤ Gr. 2. TRM was non-existent in this study.

18 patients developed AGVHD by d100 – 83% grade 1-2; 17% grade 3-4. No deaths attributed to AGVHD following study regimen.

Disease responses are: 11 (58%) patients, in relapse/active disease prior to CLO/BU, achieved CR by d30. 7 (37%) patients in CR at study entry, remained so at d30. 1 patient was N/E for disease response at d30. 1 patient (w / CLL) achieved CR at d132. 7 (37%) patients relapsed (M. d120 (d60 – d699)). 12 (60%) patients expired (M. d222 (d92 – d438)): cardiac arrest (1, d316); asp. pneumonia (1, d158); TTP (1, d438); AGVHD - post DLI (1, d175); relapse (4, M. d192 (d150 – d415)); persistent disease (1, d161); MSOF (1, d92); ITP (1, d307); Pulmonary Embolus (1, d233). Of 19 evaluable patients, 6 (32%) remain in remission with a median follow up of 946 days (31 months) (d396 – d1236).